Preparation of Fluorescein or Rhodamine Labelled Chicken Red BloodCe11s (CRBC)

Fixation

1: Obtain chicken blood commercially* in Alsever's Solution or bleed chicken from vein under wing using ACO** and 20 ml syringe (or 100 units Heparin per ml blood, can be used instead of ACO).

2. Centrifuge cells 1200 rpmand wash with PBS.

3. Resuspend cells to twice their packed volume.

4. Fix cells in 25 times their volume of .1% Glutaraldehyde for 10 minutes at room temperature.

5. .Hash .With distilled water 3X.

Conjugation to Fluorochrome

1. Chicken RBC brought to 50% solution with carbonate-bicarbonate buffer, pH 9.6

2. Add .25 m1 cell suspension to reaction mixture of choice: i
Rhodamine - 7.2 mg in 10 ml car-bicar buffer (not all dissolved)
Fluorescein - 2.4 mg in 10 ml car-bicar buffer (completely dissolved)

3. Incubate in cold overnight (~ 17 hrs).

4. Wash many times with distilled water (Rhodamine cells will still lose color)

5. Store at 7 x 108 cells/ml refrigerated.

* Bennett Labs PO Box 188
Woodland, California

** ACD = 2.2% $Na_3C_6H_5O_7 . 2H_2O$)
.8% Citric acid) to be used 1.5 m1 per 10 ml btood
2.45% Dextrose)

PBS = phosphate buffered saline .1% Glutaraldehyde is 1:500 of stock reagent